THE INFLUENCE OF ORAL AND TOPICAL CHANNA STRIATUS ON TENSILE STRENGTH, EPITHELIAZATION, FIBROBLAST COUNT AND HYDROXYPROLINE ASSAY IN LAPAROTOMY WOUND HEALING OF MALNOURISHED RATS

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Dr Syed Hassan Syed Abd Aziz:Supervisor

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Objektif: Sasaran kajian adalah untuk menilai kesan *Channa striatus* ke atas kekuatan tensil, kiraan sel epithelial dan sel fibroblast dan tahap hydroxyprolene dalam penyembuhan luka laparotomi tikus kurang zat.

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By

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Dissertation Submitted in Partial Fulfillment
Of The Requirement For The
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DISCLAIMER

I hereby certify that the work in this dissertation is my own except for the quotations and summaries which have been duly acknowledged.

Dated: ……………………………….

Dr Rezqa Abdullah Husin

PUM 0201/07

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1.0 INTRODUCTION

Traditional medicine is a comprehensive term used to refer to both systems such as traditional Chinese medicine, Indian ayurveda and Arabic Yunani medicine, and to various forms of indigenous medicine. In countries where the dominant healthcare system is based on allopathic medicine, or where traditional medicine has not been incorporated into the national health care system, traditional medicine is often termed "complementary", "alternative" or "non-conventional" medicine (WHO, 2000a).

Traditional medicine is the first point of healthcare for many people in Asia and African countries, where there has been a long and rich tradition of sourcing treatments from natural resources. The uses of natural resources in traditional medicine are mainly based from longstanding observation of the effects on the ailment throughout the generations and personal experience (WHO, 2000a). Extensive accounts of use and experiences from generation to generation provide some evidence of the effectiveness of traditional medicine. The value of traditional medicine has not been fully tested by using modern scientific means. Scientific research is needed to provide evidence of its safety and effectiveness.

Plants and animals have been used as a source of medicines from ancient times, and even in modern times, animal and plant-based systems continue to play an essential role in health care. Wild and domestic animals and their by-products (e.g., hooves, skins, bones, feathers, and tusks) form important ingredients in the preparation of curative, protective and preventive medicine. (Lev, 2003). Additionally, a significant portion of the currently available non-synthetic and/or semi-synthetic pharmaceuticals in clinical use is comprised of drugs derived from higher plants , followed by microbial, animal and mineral products, in that order (Alves RR, 2006 , Alves RR, . 2006).

The richness in animal biodiversity is still poorly explored as a good source of producing medicine despite some species is well known ingredients for some popular traditional medicine. For instance, frogs, spiders, snails and insects have been reported to produce a therapeutic effects for wound healing and curing fever or controlling blood pressure (Madan Mohan Mahawar, 2008).

In Malaysia, generally traditional medicine can be divided into 5 categories. These include Malay, Indian, and Chinese traditional medicines, homeopathy and complementary medicine. The similarity between these groups of traditional medicine is the used of similar biodiversity that are available in the same area as the source. Example is the widespread use of *Channa striatus* in Malaysia to facilitate wound healing which is the main scope of this study (Mohsin A.K, 1983a).

Channa striatus is a local freshwater snakehead fish, belonging to the *Channidae* family. It is air-breather and carnivorous fish indigenous to many tropical and subtropical countries of South America, Africa and Asia. In Malaysia, the fish is known as haruan. It is widely consumed throughout the nation not only as a food, but also as a remedy for wound healing (Mohsin A.K, 1983a).

Table 1 Some modern drugs derived from traditional medicine (Ghalib, 2007)

Drug	**Function**	**Source**	**Original Use**
Artemisinin	Antimalarial	Chinese Plant *qing hao* or sweet wormwood	Chinese traditional remedy for fevers and colds
Cromolyn	Antiasthmatic	Synthetic compound based on the khellin , the active ingredient of the plant khella	Middle Eastern traditional medicine for asthma , also used in Egypt for kidney stones
Etoposide	Antitumor	Synthesized from podophyllotoxin produced by mandrake	Various folk medicine remedies Chinese, Japanese and East
Hirudin	Anticoagulant	salivary glands of leeches , now produced by genetic engineering	Traditional remedies from around the world, from the *zhi shui* in China to European medicine in the eighteenth and nineteenth centuries.
Lovastatin	It is used to lower cholesterol	girgolas and foods such as red yeast rice . Used to synthesize other compounds such as mevastatin and pravastatin	Fungi are used to treat a broad spectrum of diseases in traditional Chinese medicine , Japanese , Eastern Europe and Russia.
Opiates	Analgesics	Seeds immature poppy	Traditional remedies Arabic, Chinese , Europeans, Indians and North Africans , used to relieve pain and treat a range of conditions such as diarrhea, cough and asthma
Quinine	Antimalarial	Cinchona bark	Traditional remedies for fever and cramps in South America
vinca alkaloids (vincristine , vinblastine)	Antitumor	Rosehip	Various folk remedies around the world , including its use as antidiabetic Jamaica, to treat wasp stings in traditional Indian medicine , eye drops, Cuba and the love potion in medieval Europe .

Many Malaysian believe that *Channa striatus* facilitate wound healing. For this reason, it is widely consumed after an injury or an operation. Women for example, during their postpartum period have *Channa striatus* as their main dish. It is also consumed by post-operative patients with the hope that it can promote and enhance healing and alleviates post-operative pain (Mat Jais, 1994).

Study has shown that *Channa striatus* has high content of essential amino acids such as glycine, aspartic and glutamine, and essential fatty acid such as arachidonic acid which are important in the process of wound healing and, as well as enhancing the antinociceptive activity (Jais *et al.*, 1998, Zuraini A, 2006). Other studies also proved *Channa striatus* enhances cutaneous wound healing (Baie and Sheikh, 2000b).

Wounds heal through several phases of haemostasis, inflammation, proliferation and remodeling (Goldsmith, 1991). Acute wound healing proceeds via unimpaired progression through those four overlapping phases. Each phase is characterized by the infiltration into the wound site of specific cell types, all of which interact and communicate, by chemical signals, to optimize repair. The healing tends to be compromised, for example, by malnutrition, infection, metabolic disturbances, or an underlying disease.

One of the wound that has huge implication on patient morbidity and mortality is the laparotomy wound. The past 50 years has witnessed a dramatic rise in therapeutic abdominal operations with improvements in perioperative critical care, advances in surgical oncology and the success of abdominal organ transplantation. The management of abdominal wall injury has in many ways not kept pace with innovations in abdominal surgery. Laparotomy incisions fail to heal 11% of the time (Pollock AV, 1989). Subsequent incisional hernia repairs fail to heal 24% to 58% of the time (Flum DR, 2003). This high rate of surgical wound failure results in thousands of reoperations and higher morbidity and mortality. In United State for example, about 4 million laparotomies performed each year, and the true incidence of laparotomy wound failure and incisional hernia formation approaches 400,000 per year. Each additional abdominal operation increases the risk of further intra-abdominal injury (Duepree HJ, 2003).

Burst abdomens, or acute fascial dehiscence with evisceration, are a rarer, but important extreme of acute wound failure. They have for a long time been associated with mortality rates of nearly 50%. It is estimated that 1% to 3% of laparotomy incisions are associated with clinically obvious dehiscence and evisceration (Carlson, 1997). Even at this relatively low rate, with approximately 4 million laparotomy incisions annually in the United States, this predicts 40,000 to 120,000 acute laparotomy wound failures each year in the United States and 20,000 to 60,000 deaths directly attributable to this dreaded wound complication. Other studies have found that more than 50% of the repaired laparotomy dehiscences will go on to form incisional hernias, entering many of these patients into a vicious cycle of surgical repair, reherniation, and acute and chronic wound complications (Hesselink VJ, 1993). Such impaired healing inflicts a huge cost upon society, diminishing the quality of life for millions worldwide.

One of the factors that affect and impair wound healing process is nutritional factor. Patients, who are malnourished or actively catabolic, such as in the systemic inflammatory response syndrome, demonstrate impaired healing. Inadequate nutrition also retards the immune response limiting opsonization of bacterial and sterilization of wounds (Demling RH, 2000). Loss of protein from protein-calorie malnutrition leads to decreased wound tensile strength, decreased T-cell function, decreased phagocytic activity, and decreased complement and antibody levels, ultimately diminishing the body's ability to defend the wound against infection (Casey J, 1983).

Malnutrition may preexist wounding or may be encountered secondary to the catabolic imbalance of the patient's overall metabolic state during wound healing. A study of orthopedic patients, including post trauma patients and patients undergoing total hip replacement, found that 42% of patients were malnourished (Jensen JE, 1982). A study conducted in 1984 where they evaluated 215 non cancer patients preoperatively found that 12% of the patients showed evidence of malnutrition (Warnold I, 1984). Another study showed that approximately 50% of all medical and surgical patients at an urban hospital in 1974 had evidence of malnutrition (Daley BJ, 1994).

The purpose of this study was therefore to investigate the healing effect of *Channa striatus* on the laparotomy wound. As malnourishment is very much prevalence among the surgical patient, this study was conducted on malnourished subjects.

2.0 REVIEW OF LITERATURE

2.1 *CHANNA STRIATUS*

Channa striatus is a snakehead fish belongs to the Channidae family. It is indigenous to many tropical and subtropical countries including Malaysia and a valuable source of protein throughout the Asia Pacific region (Mohsin A.K, 1983b). *Channa striatus* has been studied for its putative effects on wound healing (Baie and Sheikh, 2000b, Mat Jais, 1994). It is used by patients in the post-operative period to promote wound healing (Mat Jais, 1997) and to reduce pain (Zakaria, 2004).

Figure 1 *Channa striatus* species.

(Source: Google Images.net)

2.1.1 Taxonomy, Distribution and Biology

Channa striatus is a tropical, fresh water, carnivorous, air breathing fish species. *Channa striatus* come from the Channidae family, and well known as snakehead fish. There are thirty species of Channidae around the globe, and eight were found in Malaysia. The Channidae are well distributed within China, Taiwan, Indochina, Thailand, Phillippines, Indonesia and India (Mohsin A.K, 1983a).

The natural habitats of *Channa striatus* are remote water but some can be found in close proximity to settlements such as ponds, small lakes, agriculture canals, small rivers and paddy fields. Some can be found in various unexpected places such as a higher ground with water temperature about 20 C. The optimum water PH for *Channa striatus* is between 4.3 to 7.9, temperature is between 20.7 to 26.4 C, water turbidity between 2 to 268 ppm and dissolved oxygen between 1.2 to 6.1 ppm (Jais, 1991). Studies on the genetic variability of *Channa striatus*'s mitochondrial DNA revealed that *Channa striatus* being present in Malaysia for more than 600,000 years thus proving that the fish is truly Malaysian indigenous species (Jais, 2007).

Channa striatus is not a popular in the list of farming fish activity in Malaysia due to its carnivorous behaviour (Mohsin A.K, 1983a). However, some countries in the region, such as Indonesia, Thailand and Indochina are having extensive breeding programs because it is among the popular table dishes in seafood restaurants (Jais, 1991) .

2.1.2 Chemistry of *Channa striatus*

Several studies have been done recently to analyze and identify the main composition of *Channa striatus.* It mainly consist of protein (78.32 +-0.23%). The aqueous extract of *Channa striatus* was found to contain all amino acids, with the major amino acids found being glycine, alanine, lysine, aspartic acid and proline (35.77%, 10.19%, 9.44%, 8.53% and 6.86 % of total protein, respectively) (ZA Zakaria, 2007).

Channa striatus lipid content is quite low at 2.08 %. It also has a high content of arachidonic acid and docosahexaenoic acid (DHA). The most abundant fatty acid present in the aqueous extract of *Channa striatus* is palmitic acid (C16: 0), which accounted for approximately 35.93 % of total fatty acids. The other major fatty acids included oleic acid (C18: 1), stearic acid (C18: 0), linoleic acid (C18: 2) and arachidonic acid (C20: 4), which accounted for the 22.96%, 15.31%, 11.45 % and 7.44 % of total fatty acids, respectively. *Channa striatus* was also found to have ratios of w-3 : w-6 and polyunsaturated fatty acid:saturated fatty acid (PUFA/SFA) lower than 1 (Zuraini A, 2006, ZA Zakaria, 2007).

Channa striatus also has good amount of dietary mineral such as magnesium, calcium, copper, manganese, iron and zinc. Nickel and lead also naturally occurring mineral in *Channa striatus* but they are well below toxic level to human (jais, 1997).

Table 2 Protein composition of Channa striatus (Jais *et al.*, 1998)

Amino acid	**Total Protein %**
Aspartic acid	8.53
Glutamic acid	4.59
Serine	3.40
Glycine	35.77
Histidine	1.61
Arginine	4.09
Theorine	4.07
Alanine	1.19
Proline	6.86
Tyrosine	1.10
Valine	2.18
Methionine	1.53
Isoleucine	1.28
Leucine	2.91
Phenylalanine	2.48
Lysine	9.44

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Table 3 Fatty acid composition of Channa Striatus (Jais *et al.*, 1998)

Fatty acid	% Total fatty acid
Myristic acid	2.15
Palmitic acid	35.93
Stearic acid	15.31
Heptadecanoic acid	2.90
Palmitoleic acid	1.86
Oleic acid	22.96
Linoleic aicd	11.45
Arachidonic acid	0.83

2.1.3 Antimicrobial Properties of Channa striatus

Channa striatus's extract had shown positive although mild results as anti-bacterial and anti-fungal. *Channa striatus's* extract has shown inhibition effects on the growth of 13 filamentous fungus and 3 non-filamentous or yeast species (Mat Jais, 2007).

2.1.4 Antinociceptive Activity of *Channa striatus*

The *Channa striatus* fillet extract produces a dose dependent anti-nociceptive property, which is also essential in healing process (Mat Jais, 1997). Based on studies on rat, Channa striatus extract was found to have comparable result to opiate in analgesic properties as well as enhancing action of opiate. Further studies on effect of pH and heat to its nociceptive properties showed that Channa striatus maintains its activity within pH range of 6.0 to 8.0 and minimal loss of antinociceptive activity to heat. Study on naloxone given pre treatment had no effect on the activity of the extract of Channa striatus (Dambisya *et al.*, 1999, Mat Jais *et al.*, 1997).

2.1.5 *Channa striatus* on Skin Disease

Channa striatus has been proven to be useful in skin related illness as to maintain a good healthy skin via the action of DHA (docosahexanoic acid) (Mat Jais AM, 1998, Mori T, 1999). This has been identified as a nutraceutical with clinical value in treating skin related problems. In psoriasis, AA (arachidonic acid) metabolism is altered due to the action of 12-HETE (12-hydroxyeicosatetraenoic acid) resulting in a reducing level of leukotriene B4 (substances for pro-inflammatory lipoxygenase products – involved in maintaining of chronic topical inflammatory conditions (Rao TS, 1994).

2.2 THE SKIN

2.2.1 General Anatomy and Histology of the Skin

Human skin consists of layers of stratified, cellular epidermis and an underlying dermis of connective tissue. The dermal–epidermal junction is undulating in section; ridges of the epidermis, known as rete ridges, project into the dermis. The junction provides mechanical support for the epidermis and acts as a partial barrier against exchange of cells and large molecules. Below the dermis is a fatty layer, the panniculus adiposus, usually designated as 'subcutaneous'. This is separated from the rest of the body by a vestigial layer of striated muscle, the panniculus carnosus (Quinn, 2004).

The superficial epidermis is a stratified epithelium largely composed of keratinocytes that are formed by division of cells in the basal layer, and give rise to several distinguishable layers as they move outwards and progressively differentiate. Within the epidermis, there are several other cell populations, namely melanocytes, which donate pigment to the keratinocytes , Langerhans' cells, which have immunological functions and Merkel cells (Quinn, 2004).

The hair follicles comprise pockets of epithelium that are continuous with the superficial epidermis. They undergo intermittent activity throughout life. During the active phase, the follicle envelops at its base a small papilla of dermis (Venus *et al.*, 2010). A bundle of smooth muscle, the arrector pili, extends at an angle between the surface of the dermis and a point in the follicle wall. Also derived from the epidermis, and opening directly to the skin surface, are the eccrine sweat glands, present in every region of the body in densities of 100–600/cm2 .

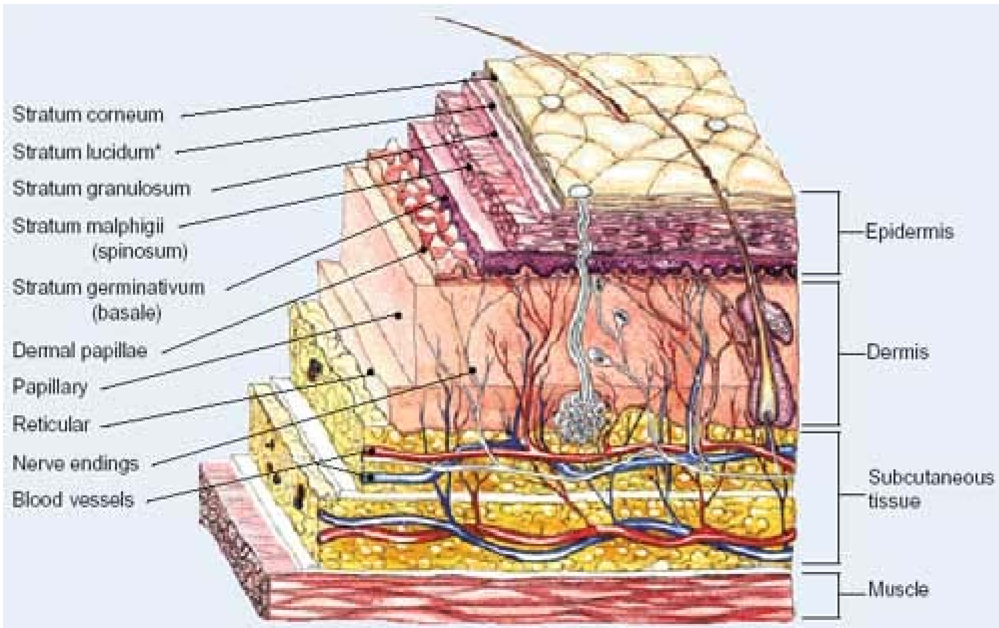

Figure 2 Structure of the skin (source: http://factoidz.com/structure-and-function-of-the-skin/)

The basis of the dermis is a supporting matrix or ground substance in which polysaccharides and protein are linked to produce macromolecules with a remarkable capacity for retaining water. Within and associated with this matrix are two kinds of protein fibre: collagen, which has great tensile strength and forms the major constituent of the dermis, and elastin, which makes up only a small proportion of the bulk.

The cellular constituents of the dermis include fibroblasts, mast cells and histiocytes (monocyte/macrophages). The dermis has a very rich blood supply, although no vessels pass through the dermal–epidermal junction.The motor innervation of the skin is autonomic, and includes a cholinergic component to the eccrine sweat glands and adrenergic components to both the eccrine and apocrine glands, to the smooth muscle and the arterioles and to the arrector pili muscle. The sensory nerve endings are of several kinds: some are free, some terminate in hair follicles and others have expanded tips (Goldsmith, 1991, Montagna W, 1992, J.A. McGrath, 2010).

2.3 WOUND

A wound is defined as a separation or discontinuity of the skin, mucous membrane or tissue caused by physical, chemical or biological insult (Roberts, 1996). This separation or discontinuity causes the loss of protective barrier of the skin from the elements. Some of the skins or tissues could be lost in the process of wounding with or without lost of underlying connective tissues (e.g muscle, soft tissue, bones or nerves). The cause of the injury could be anything for example wound following surgery, blunt trauma, cut, chemicals, heat, cold, friction, pressure or result of disease (e.g. leg ulcers or carcinomas) (Roberts, 1996).

2.3.1 Wound Classification

There is no single, standard classification used for wound. However, there are a number of different ways in which wounds can be classified. The different systems of classification are complementary to each other, thus a comprehensive description of a wound requires the use of more than one system of classification (Enoch, 2004).

Good classification is important in aiding diagnosis and stratification, ensuring uniformity of documentation, offering prognostic information and in guiding the management (Kumar and Leaper, 2008).The factors of great importance in wound classification are the nature of the injury causing the wounds, the timing of the injury, acute or chronic injury, and the depth of the injury in relation to the skin and underlying tissues and tissues lost (Franz *et al.*, 2007). These factors will exert a significant effect on the ability of the wound to heal and give information on the best way to manage the wound for optimal healing.

Wound can generally be classified as (Flanagan, 1994):

- Superficial wound (loss of epidermis only)
- Partial thickness wound (involve the epidermis and dermis)
- Full thickness wound (involve the epidermis, dermis, subcutaneous fat and sometimes bone)

Wound can further be classified according to aetiology, morphology, degree of contamination and complexity.

Table 4 Classification of wound (Kumar and Leaper, 2008)

Aetiology	Morphology	Contamination	complexity
Surgical Penetrating trauma Stab Projectile injury Bite/envenomation Blunt trauma Avulsion/traction Crush injury Burns Thermal Electrical Irradiation Frost-bite	Abrasion Incision Superficial Deep Laceration Superficial Deep Degloving injury Ulceration Superficial Deep	Clean Implant Non-implant Clean-contaminated Contaminated Dirty-infected	Simple Complex Open fracture Laparostomy Complicated Wound infection Gas gangrene Flap necrosis

Table 5 Wound classification according to degree of contamination (Surgeon, 2006)

Class	**Infection rate (%)**	**Wound Characteristics**
Clean (Class I)	1.5-5.1	Atraumatic, uninfected; no entry of urinary, GI or respiratory tract.
Clean-contaminated (Class II)	7.7-10.8	Minor breaks in sterile technique, entry of urinary, GI or respiratory tracts without significant spillage.
Contaminated (Class III)	15.2-16.3	Traumatic wounds; gross spillage from GI tract; entry into infected tissue, bone, urine or bile.
Dirty (Class IV)	28-40	Drainage of abscess; debridement of soft tissue infection.

2.4 WOUND HEALING

The wound-healing process is a complex series of events. It begins immediately after the occurrence of an injury and the process of wound healing can continue for months even up to years. The ultimate end of this process is the restoration of an intact epithelial barrier with the maximum strength that it could achieve and serve it functions (Roberts, 1996).

The wound healing process is a dynamic one. It requires various cellular mechanisms and chemical mediators for successful completion. It can be divided into three phases, of which require specific conditions and requirements. These phases are not exclusive but overlapping in time and effect to each other. It is not a linear process and can progress forward and backward through the phases (Wild *et al.*, 2010). A successful healing requires adequate blood supply and nutrients to the site of damage (Stadelmann W.K, 1998). The speed at which they develop depends on the potential for regeneration and repair of the tissue affected. Skin for example has the ability to rapidly heal in comparison to the bone.

2.4.1 Phases of Wound Healing

The three phases of the wound healing are (Hutchinson 1992):

1. Inflammatory phase
2. Proliferation phase
3. Maturation and remodelling phase

2.4.2 Inflammatory Phase

The inflammatory phase is regarded as the first phase of wound healing and characterized by its cardinal signs: rubor (redness), calor (warmth), tumor (swelling), dolor (pain), and functio laesa (loss of function). The aims of this phase are to stop the bleeding, seal the surface of the wound, and remove any necrotic tissue, foreign bodies, or bacteria. These are achieved by increased in the vascular permeability, migration of cells into the wound by chemotaxis, and the secretion of cytokines and growth factors (Leaper, 2008).

Immediately after acute injury, haemostatic mechanisms and pathways commence. Initial intense local vasoconstriction of arterioles and capillaries is followed by vasodilatation and increased vascular permeability. Blood constituents spill and platelets are exposed to types IV (basement membranes) and V (skin) collagen. Platelets are activated and release an array of soluble inflammatory mediators (Goldsmith, 1991). The intrinsic and extrinsic arms of the coagulation cascade are activated, resulting in the deposition of fibrin nets to trap blood cells, forming the clot, and seal the wound. The fibrin nets work as a scaffold for endothelial cells, inflammatory cells, and fibroblasts (Enoch, 2004).

In addition to establishing haemostasis, platelets release various soluble factors that attract and activate inflammatory cells. Histamine and serotonin increase the permeability of local capillary beds, allowing leukocytes to infiltrate the wound. Neutrophils arrive early and clean the area of bacteria and foreign particles. Migration of neutrophils stop when wound contamination has been controlled, usually within the first few days after injury. They do not survive longer than 24 hours. However, if wound contamination persists, there is a sustained influx of neutrophils into the wound. Macrophages replace neutrophils as the predominant cells in the wound by two days after injury and they phagocytize bacteria and damaged tissue. The macrophages also release matrix metalloproteinases, which degrade the extracellular matrix and are crucial for removing foreign material, promoting cell movement through tissue spaces, and regulating extracellular matrix turnover. Macrophages also secrete a number of factors such as growth factors and other cytokines, especially during the third and fourth post-wounding days. They promote angiogenesis, stimulate cells that reepithelialise the wound, create granulation tissue, and lay down a new extracellular matrix pushing the wound healing process into the next phase (Enoch, 2004, Quinn, 2004).

2.4.3 Proliferative Phase

This phase is also known as reconstructive phase where the tissues loss will be replaced. It involves angiogenesis, fibroblast and granulation tissues formation, collagen deposition, epithelisation and contraction (Leaper, 2008).

The proliferative phase begins with the formation of a provisional matrix of fibrin and fibronectin as part of initial clot formation. Initially, the provisional matrix is populated by macrophages; however, by day 2, fibroblasts appear in the fibronectin fibrin framework and initiate collagen synthesis, produce glycosaminoglycan (hyaluronic acids) and proteoglycan (Leaper, 2008).

There are many type of collagen. In the skin, collagen type I consist of 80% of the collagen in skin, and type III makes up the remaining 20%. A higher percentage of type III collagen is seen in embryologic skin and in early wound healing. An important aspect of collagen synthesis is the hydroxylation of lysine and proline moieties within the collagen molecule. This process requires specific enzymes as well as oxygen, vitamin C, ketoglutarate, and ferrous iron, which function as cofactors (Enoch, 2004).

Hydroxyproline, which is found almost exclusively in collagen, serves as a marker of the quantity of collagen in tissue. Hydroxylysine is required for covalent cross-link formation between collagen molecules, which contributes greatly to wound strength. After collagen molecules are synthesized by fibroblasts, they are released into the extracellular space and align themselves through enzymatic reaction into fibrils and fibers that give the wound strength (Okasala and Inki, 1995).

In addition to glycosaminoglycans, proteoglycans and collagen, fibroblast generate cytokines such as PDGF, tumor growth factor-B, bFGF, keratinocyte growth factor, and insulin-like growth factor-1. Fibroblasts also assemble collagen molecules into fibers, which are cross-linked and organized into bundles. Hence, collagen is the major component of acute wound connective tissue, with net production continuing for the next 6 weeks. The growing content of wound collagen correlates with an increasing tensile strength (Wild 2010).

Angiogenesis occurs concurrently with fibroblast when endothelial cells migrate to the area of the wound. Because the activity of fibroblasts and epithelial cells requires oxygen and nutrients, angiogenesis is important for other stages in wound healing, like epidermal and fibroblast migration. The tissue in which angiogenesis has occurred typically looks red due to the presence of capillaries. The formation of granulation tissue in an open wound allows the reepithelialization phase to take place, as epithelial cells migrate across the new tissue to form a barrier between the wound and the environment (Enoch, 2004).

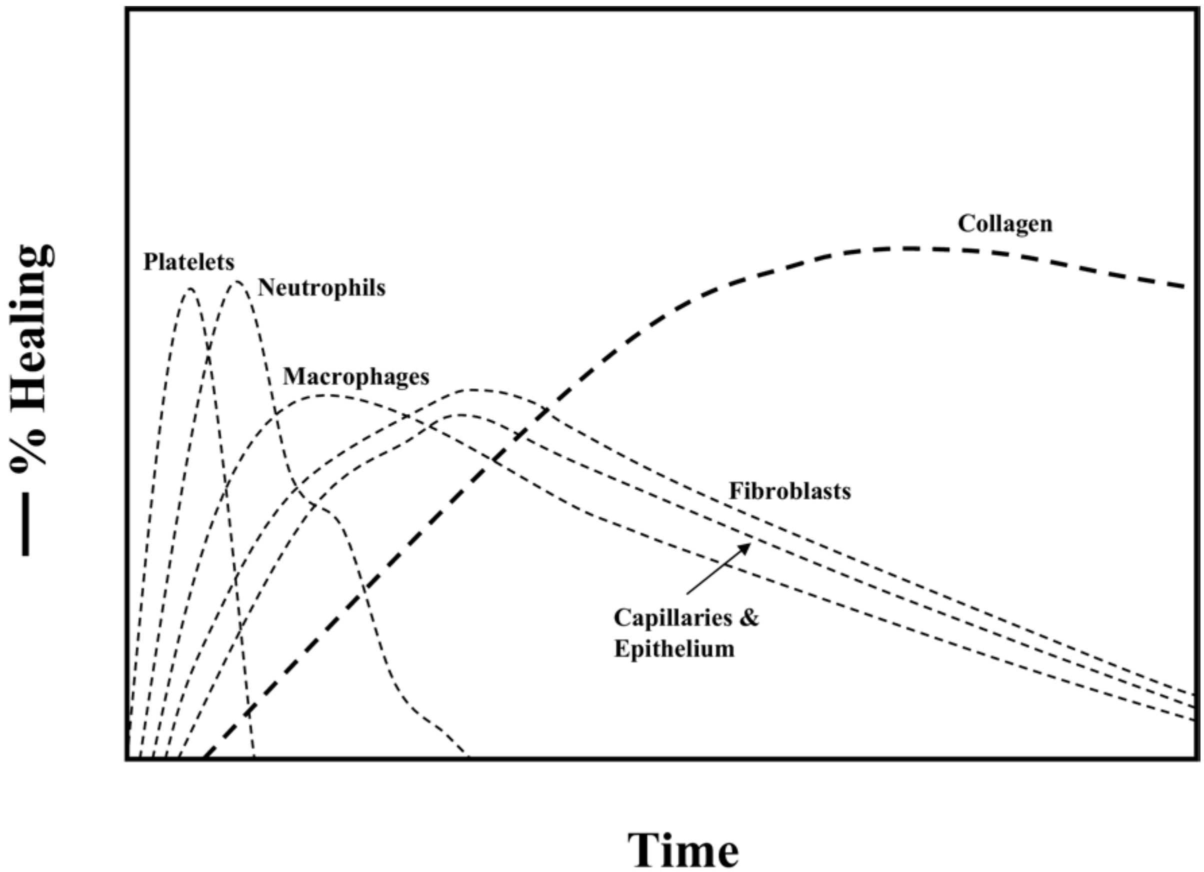

Figure 3 The mechanism of acute wound healing. Acute wounds predictably activate normal cellular and olecular processes and pathways to achieve progressive and timely tissue repair. (Franz MG, 2009).

Contraction commences approximately a week after wounding, when fibroblasts have differentiated into myofibroblast. In full thickness wounds, contraction peaks at 5 to 15 days post wounding. Contraction can last for several weeks and continues even after the wound is completely reepithelialized. A large wound can become 40 to 80% smaller after contraction (Franz *et al.*, 2007).

2.4.4 Maturation and Remodelling Phase

Maturation phase is the final phase and occurs once the wound has closed or already healed. This phase begins at approximately day seven after surgery and last for one year or more.

The transition from the proliferative phase to the maturation phase is defined by reaching collagen equilibrium (Enoch, 2004). Collagen accumulation within the wound reaches a maximum within 2 to 3 weeks after wounding. Tensile strength gradually increases as random collagen fibrils are replaced by organized fibrils with more intermolecular bonds. Type I collagen replaces type III collagen until the normal skin ratio of 4:1 is achieved. Continued collagen deposition and remodeling contribute to the increased tensile strength of wounds. The dense bundle of fibres, characteristic of collagen, is the predominant constituent and form the basis of the formation of scar. Cellular activity also reduces and the number of blood vessels in the wounded area begin to regress and decrease. Tensile strength plateaus at 80% of the original strength approximately 1 y after injury. Scar tissue never reaches the tensile strength of unwounded tissue (Franz *et al.*, 2007).

2.4.5 Wound Closure

Wound closure can be divided into three depending on the timing and method by which wound closure is achieved (Roberts, 1996).

Primary wound closure refer to a wound which, when mechanically closed by approximation of wound edges, heals without complications at the first instance. Advantages of primary closure are good cosmetic effect and shorter time to clinical healing. Primary closure is the preferred method of closure for clean wounds with minimal tissue loss. Primary closure should ideally be performed within 6–8 hours of trauma. Biochemical process in primary healing is mainly by deposition of connective tissue (Suzanne K Doud Galli, 2010, Suzanne K, 2010).

Secondary wound closure or healing by secondary intention occurs when a wound which is left open heals largely by formation of granulation tissue and contraction. Wounds that are candidates for secondary closure include those with significant tissue loss precluding tension-free approximation of edges, devitalized edges, ulcerations, and abscess cavities. These wounds heal more slowly due to the amount of connective tissue that is necessary to fill the wound (Suzanne K, 2010).

Delayed primary closure is the delayed closure of a wound after a variable period of time for which it has been left open. It is a combination of the other two types of wound healing. In general, tertiary closure is applied to wounds that are grossly contaminated or